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Organic Chemical Bulletin

Volume 32 • 1960 • Number 3

Address inquiries to
Eastman Organic Chemicals Dept.
Distillation Products Industries
Division of Eastman Kodak Company
Rochester 3, N. Y.

PUBLISHED BY THE RESEARCH LABORATORIES OF THE EASTMAN KODAK COMPANY

FRACTIONATION BY COUNTERCURRENT EXTRACTION

By MARTHA A. HILL

With vastly improved apparatus now available, countercurrent fractionation has advanced from a novelty to a position of recognized importance among laboratory techniques for the resolution of chemical mixtures. Typical applications are: establishing degree of purity, performing analytical separations, and recovering the products of chemical syntheses. Theory and practice are discussed in hundreds of papers, mostly published during the last ten years. One of the most helpful is an authoritative review and bibliography by Lyman C. Craig (1).

Apparatus

A countercurrent extraction, or distribution, is really a succession of solvent-solvent extractions in a multiple-tube apparatus which permits one of the solvent phases to advance to the next tube of the series independently of the other phase. A number of commercial models of extraction trains are available, some with banks of 100 or more tubes and with optional programming devices to control the operation from start to finish. An example is the Craig-Post apparatus (2). A mobile unit which is more compact has been described by Raymond (3).

Automatic pipetting devices are recommended for the filling operation with instruments that do not already have this feature.

Fundamental Countercurrent Procedure

It is convenient to think of the fractionator as having a bank of separatory tubes in which the lower solvent phase remains stationary when the upper phase is decanted into the next tube in the series. The sample is dissolved and equilibrated with measured volumes of two immiscible solvents in the first tube, conveniently designated as tube 0. A gentle rocking motion suffices for good mixing. The layers are permitted to separate and the upper phase is decanted into an adjacent separatory tube (tube 1) where it comes in contact with an equal volume of fresh, lower-phase solvent. Simultaneously, an equal volume of upper-phase solvent is added to the residual solution in tube 0. The tubes are then rocked to establish equilibrium, the layers are allowed to separate, and a second transfer is effected. This time the upper layer from tube 1 goes into tube 2 (containing fresh, lower-phase solvent) while that from tube 0 advances into tube 1. Always adding fresh solvent to replace

the upper phase decanted out of tube 0, the mixing-and-transfer sequence is continually repeated until the desired degree of separation has been accomplished or until the original upper layer from tube 0 has advanced to the last tube in the train.

Analysis of Fractions

After the final transfer, the contents of the individual tubes are collected and analyzed by any convenient method. If enough solute is present, the fractions may be evaporated to dryness and weighed in tared glass or aluminum shells.* An alternative method, useful in many instances, is to measure spectral absorbance. In this case, all the solute is recovered in one liquid phase, either by "salting out" or by adding a third solvent (e.g., an alcohol) to make the layers miscible, then spectra are recorded over the regions of known absorbance. The data provide qualitative as well as quantitative information about the fractions. Scanning a series of 50 or even 100 solutions is less laborious than might be supposed, if there is a recording spectrophotometer available. Used in conjunction with automatic sampling devices, which can be arranged to transfer the fractions to and from the measurement cell of an automatic photometer, an astonishing amount of data can be accumulated with a minimum investment of the operator's time.

The data (concentration, maximum absorbance, etc.) are plotted against tube number. A symmetrical distribution curve with only one peak indicates a pure material; techniques are available for detecting and interpreting even minor deviations from symmetry (4, 5). Figure 1 is a typical curve, obtained in a 25-tube

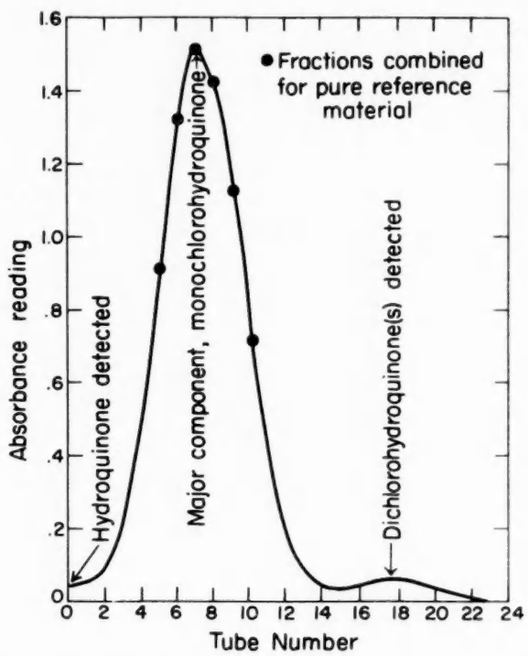

Fig. 1. Purification of monochlorohydroquinone by countercurrent extraction: stationary phase solvent, 0.5% in water; advancing phase solvent, petroleum ether (7 parts) + ethyl ether (3 parts).

fractionation of commercial-grade chlorohydroquinone. Hydroquinone and dichlorohydroquinone(s) were detected, along with other, unidentified impurities in fractions taken from tubes 0 and 19, respectively. The high-quality monochlorohydroquinone recovered from tubes 5-10 was used as the infrared reference material in establishing ASA Standard PH 4.134—1956.

*Aluminum-foil dishes of about 50-ml. capacity are convenient, and sufficiently inexpensive to be discarded after one use.

Single Withdrawal Procedure

The fractionator may be operated beyond the usual number of cycles by arranging to collect the upper layers as they emerge from the last tube in the series. Two distribution curves are obtained—one for the effluent fractions and the other for the fractions remaining in the apparatus after the last transfer has been completed. This technique has particular value in separating materials whose partition ratios are less than 1.

Recycling Procedure

In the fractionation procedures just described, fresh solvent is decanted from a reservoir into tube 0 during each transfer step, and the upper layer (if any) from the last tube is led off into a fraction collector. For reasons of economy as well as convenience, it is sometimes preferable to operate the fractionator as a closed system. All tubes are charged with upper- as well as lower-phase solvent, then the first and last tubes in the train are connected so that an upper layer emerging from the final tube is conducted back into tube 0 and recycled.

Choice of Solvents

A mixture of two or more solvents is required that will separate easily into two immiscible layers. Metzsch (6) lists about 400 nearly-immiscible pairs. Low viscosity and marked differences in relative density and dielectric constant are helpful qualifications, along with a tendency to resist forming stable emulsions. Availability, toxicity, stability, boiling point, and spectral transparency are important secondary considerations. Both layers should be capable of dissolving at least moderate amounts of the sample without permanently altering it. The use of modifying salts, buffers, or complexing agents often helps in achieving a favorable partition ratio. Partition ratio, K, is the ratio of equilibrium concentrations (C) of solute in the two layers.

$$K = C_u / C_l = \frac{F_u}{V_u} \times \frac{V_l}{F_l},$$

where subscripts u and l refer to the upper and lower phases, respectively, F = fraction of solute in a given phase, and V = volume of a given phase. Figure 2 shows the effect of a fourfold change in partition ratio on the distribution of solute in a 100-transfer experiment when equal volumes of upper- and lower-phase solvents are used.

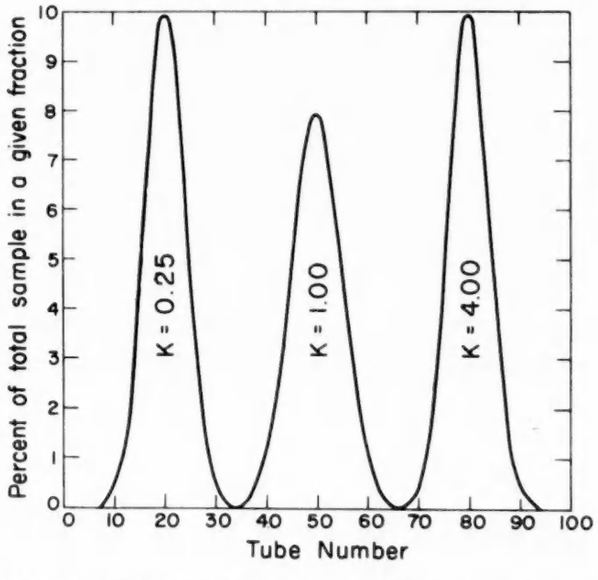

Fig. 2. Effect of 4-fold change in partition ratio on distribution of solute in a 100-transfer experiment.

EXAMPLE: In a 100-tube distribution where $K = 1$, and therefore expression $\left[\frac{2nK}{(K+1)^2}\right]$ equals 50:

(a) The percentage in the peak tube (y = 0):

$$y_o = \frac{100}{\sqrt{3.14 \times 50}} \cdot e^0 = 7.98\%$$

(b) The percentages in the neighboring tubes are:

$$y_1 = y_{-1} = y_o \cdot e^{-1/50} = 7.82\%$$
$$y_2 = y_{-2} = y_o \cdot e^{-4/50} = 7.37\%, \text{ etc.}$$

4. Determining sample purity by graphical method:

$$\log D - \log \frac{n!}{r!\,(n-r)!} = \log D_t - n \log (K+1) + r \log K, \qquad (6)$$

where

D = amount of solute in a given tube, r, expressed in any convenient units (weight, spectral absorbance, etc.); D_t = total amount of solute in all tubes of distribution, $\Sigma D_1 + D_2 + D_3 + \ldots\ldots D_n$; n = number of tubes in the distribution; and K = partition coefficient (applies to systems with equal phase volumes).

Treating Equation (6) like the equation for a straight line ($y = a + bx$), observed values for $\left[\log D - \log \frac{n!}{r!\,(n-r)!}\right]$ are plotted *vs.* r values.

(a) If the plot yields a single, straight line, the sample is pure. The slope of the line is log K for the pure compound.

(b) If the curve has more than one straight-line region, each section represents one component that has been isolated. As before, the slope is log K. The intercept of the line extrapolated to $r = 0$ is $[\log D_t - n \log (K+1)]$, from which may be calculated the total amount (D_t) or percentage of the given component in the original sample.

(c) Curved sections of the plot correspond to unresolved mixtures of two or more components.

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